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# Extended-Spectrum Beta-Lactamase-Producing Escherichia coli and *Klebsiella pneumoniae* among Chickens, Poultry Environment and Retail Meats in Owerri Nigeria

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## Abstract

The carriage of extended-spectrum beta-lactamase (ESBL) producing bacteria in food-producing animals and contamination of retail meat may contribute to increased incidences of infections with ESBL-producing bacteria in humans. This study investigated the occurrence of ESBL genes among *Escherichia coli* and *Klebsiella pneumoniae* isolates from poultry and retail meat sources in Nigeria. Bacteria were cultured and identified using standard microbiological methods. Phenotypic detection of ESBL was done using the double-disk synergy test while PCR was used for the detection of ESBL genes. Out of 272 samples, a total of 172 (63.2%) non-duplicate bacterial isolates were identified as *E. coli* and *K. pneumoniae.* The prevalence rate of these isolates was 62.8% (108/172) for *E. coli* and 37.2% (64/172) for *K. pneumoniae* respectively. The highest resistance rate was observed against cefotaxime (70.9%), followed by amoxicillin-clavulanic acid (66.3%) and ceftazidime (65.1%). There was no significant difference in the rate of resistance between *E. coli* and *K. pneumoniae* $P > 0.05$. Out of the 172 bacterial isolates, 16.9% (29/172) were phenotypically characterized as ESBL producing isolates. PCR showed that all the 12 isolates subjected to the assay harbored at least one ESBL gene; $bla_{CTX-M}$, $bla_{TEM}$ or $bla_{SHV}$. The most prevalent ESBL gene was $bla_{CTX-M}$ detected in 91.7% (11/12) of the isolates, followed by $bla_{TEM}$, 75% (9/12), and $bla_{SHV}$, 66.7% (8/12) respectively. These findings suggest a high prevalence of ESBL genes in the food chain. Surveillance along the poultry production-meat-consumer chain would be a significant tool to identify sources of emerging multidrug-resistant pathogens in Nigeria.

***Keywords:*** *ESBL; Escherichia coli; Klebsiella pneumoniae; Nigeria*

## Introduction

The Enterobacteriaceae resistance to extended-spectrum cephalosporins mostly caused by Extended-Spectrum beta-lactamases enzymes (ESBLs); constitutes a major health challenge in both veterinary and human medicine. These plasmid-borne ESBL genes can be easily transferred between and within bacterial species. The infections caused by ESBL producing Enterobacteriaceae were originally a hospital-related problem with the acquisition in hospitals or associated healthcare contact. However, in the past two decades, this has changed, with people who had no healthcare contact also being rectal carriers of ESBL producing Enterobacteriaceae [1,2]. The one-health approach comprising humans, animals, and the environment as an interconnected entity has been adopted to carry out studies aimed at unraveling the reservoirs and routes of transmission of antimicrobial-resistant microorganisms and resistance genes.

Poultry and contaminated retail meat have been suggested as a potential source for ESBL producing Enterobacteriaceae and there is increasing evidence for the transmission of ESBL genes from these sources to humans, most likely through the food chain [3]. In Denmark and the Netherland, similar or identical ESBL producing isolates were found in chicken meat and patients [4,5]. Another study has shown that ESBL-producing *E. coli* causing extraintestinal infections in humans originated from meat products, particularly poultry (Lazarus., *et al*. 2014). Also, Liu., *et al*. [6] showed that poultry meat can act as a vehicle for exposure and infection with a specific *E. coli* strain ST131 sublineages. These have necessitated the setting up of various national and international guidelines that regulate and prevent the inappropriate use of antimicrobials in food animal production.

In Nigeria, antibiotics are readily available for use in poultry production, either for therapeutic or preventive purposes and unlike in developed countries, antibiotic-resistant Enterobacteriaceae from poultry is not sufficiently monitored and the use of antibiotics remains largely unregulated in the industry [7]. The unprecedented ease with which poultry products are being traded globally has made the control of antibiotics resistance a big challenge in a developing country such as Nigeria, where surveillance systems are poorly implemented. In this study, we investigated the prevalence of ESBL producing *E. coli* and *K. pneumoniae* isolates in the food production chain using samples from Chicken faeces, poultry environment, and retail meat in Owerri southeast Nigeria. This is a part of an ongoing study to generate baseline data for the implementation of antibiotics resistance action plan in the region using one health approach.

## Materials and Methods

Cochran's formula was used to determine the sample size used in this study. A total of 272 samples were collected; including fresh fecal samples from chickens that had not contacted the soil (n = 52), poultry environmental samples such as litter and water randomly collected from different locations on the poultry farms (n = 134) and retail meat viz: beef, chicken, and goat meat (n = 86). The samples collected from March to July 2021 came from three different poultry farms and a major market all located in Owerri Nigeria. The Ethics Committee of the Federal University of Technology Owerri Nigeria approved the study protocol. All sampling procedures were per the guidelines of the National Health Research Ethics Committee, Nigeria (https://nhrec.net).

### Isolation, characterization and identification of *Escherichia coli* and *Klebsiella pneumoniae*

Standard microbiological techniques including culture, biochemical assay, and microscopy were adopted for the isolation and identification of bacteria [8]. Samples were first inoculated into an enrichment medium (peptone water) overnight before subsequent streaking onto MacConkey lactose agar. All incubations were done at $37^0$C for 24hrs.

### Antibiotics susceptibility testing

The antibiotics susceptibility pattern of the isolates was determined by the Kirby-Bauer disk diffusion method against a panel of 10 antimicrobial agents. Briefly, 3 - 5 colonies of the test isolates were picked by sterile wire loop and transferred to a sterile normal saline in a test tube. The isolates were standardized by adjusting to 0.5 McFarland turbidity standards; using a sterile cotton swab, the isolates were evenly spread on Muller Hinton agar plate. After about 3 - 5 minutes, the antibiotics disks were placed on the plates and incubated overnight at $37^0$C. The zone of inhibition was measured and interpreted as susceptible (S) and resistant (R) using the Clinical Laboratory Standard Institute breakpoint [9]. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were included as quality control strains. The following antibiotics were tested: Imipenem (IPM), cefpirome (CEF), ceftazidime (CAZ), cefoxitin (FOX), cefotaxime (CTX), amoxicillin-clavulanic acid (AMG), erythromycin (E), moxifloxacin (MFX) and chloramphenicol (C), gentamycin (CN).

### Phenotypic detection of ESBLs

Phenotypic detection of ESBL was carried out using Double-Disk Synergy Test (DDST) method. A sterile cotton swab was used to spread the standardized isolates on Mueller Hinton agar plates; single discs of ceftazidime (30 µg), cefotaxime (30 µg), and cefpirome

(30 µg) were then placed 50 mm center to center from the amoxicillin-clavulanic acid disc (20 µ/10 µg) on MH plates. The plates were incubated at 37$^0$C overnight. Enhanced zones of inhibition by ≥ 5 mm between any of the beta-lactam discs and the amoxicillin-clavulanic acid disc were recorded as ESBL producers according to the CLSI [10] guidelines.

### PCR detection of ESBL genes

Genomic DNA was extracted using Quick-DNATM Miniprep Plus Kit (Catalog Nos. D3024 and D3025), according to the manufacturer's protocol. The PCR was conducted with a total volume of 25 µl containing 12.5 µl of One Taq Quick-Load 2X Master Mix with Standard Buffer (New England Biolabs Inc.); 0.5 µl each of forward and reverse primers; 8.5 µl of Nuclease free water and 3 µl of DNA template was used for PCR amplification of ESBL genes. The primers TEM, SHV and CTX were used with amplification conditions as shown in table 1. Agarose gel electrophoresis was carried out using 1.5% agarose gel with ethidium bromide for 1h at 80V.

| Primer | Primer Sequence (5'- 3') | Amplification condition | Amplicon product size (bp)/Ref |
|---|---|---|---|
| $bla_{TEM}$ (forward)<br>$bla_{TEM}$ (reverse) | TCAACATTTCCGTGTCGCAC<br>AGTTACCAATGCTTA | Initial denaturation: 94°C, 5 minutes<br>Denaturation: 94°C, 30 seconds<br>Annealing: 55°C, 1 minute<br>Extension: 72°C, 2 minutes<br>Final extension: 72°C, 5 minutes | 861<br>Zong., *et al*. [11] |
| $bla_{CTX-M}$ (forward)<br>$bla_{CTX-M}$ (reverse) | CGCTTTGCGATGTGCAG<br>ACCGCGATATCGTTGGT | Initial denaturation: 94°C, 5 minutes<br>Denaturation: 94°C, 30 seconds<br>Annealing: 55°C, 1 minute<br>Extension: 72°C, 2 minutes<br>Final extension: 72°C, 5 minutes | 544<br>Zong., *et al*. [11] |
| $bla_{SHV}$ (forward)<br>$bla_{SHV}$ (reverse) | CGCCTGTGTATTATCTCCCT<br>CGAGTAGTCCACCAGATCCT | Initial denaturation: 94°C, 5 minutes<br>Denaturation: 94°C, 30 seconds<br>Annealing: 54°C, 30 seconds<br>Extension: 72°C, 1 minutes<br>Final extension: 72°C, 10 minutes | 445 bp<br>Pitout., *et al*. [12] |

***Table 1:*** *Primer sequences and their amplification condition.*

### Statistical analysis

Data were presented as descriptive frequencies. Statistical analysis was performed using SPSS, version 21.0 (Armonk, NY, USA). The comparison of variables was done by Fisher's exact test and the p-value less than 0.05 was considered as a statistically significant association.

## Results

### Distribution of the bacterial isolates in the poultry and meat samples

A total of 272 samples were analyzed in this study; including chicken faeces (n = 52), poultry environment (n = 134), and retail meat (n = 86). From these 272 samples, a total of 172 (63.2%) non-duplicate bacterial isolates were identified as *E. coli* and *K. pneumoniae*. The prevalence rate of these isolates was 62.8% (108/172) for *E. coli* and 37.2% (64/172) for *K. pneumoniae* respectively. Among the 108 *E.*

*coli* isolates, the highest number came from meat samples, 43.5% (47/108) followed by poultry environment and chicken faeces 40.7% (44/108) and 15.7% (17/108) respectively. Similarly, out of the 64 *K. pneumoniae* isolates obtained; 37.5% (24/64) came from Chicken faeces while 32.3% (20/64) each came from poultry environment and meat samples respectively (Figure 1). Among the 172 isolates, 41 bacterial isolates came from chicken faeces sample, 64 from poultry environment, and 67 from meat samples (Table 2).

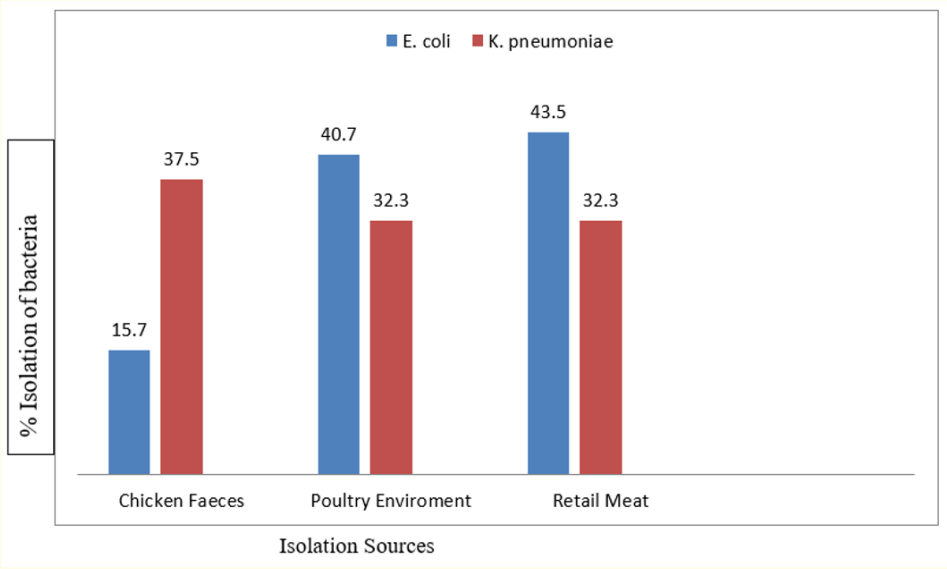


***Figure 1:*** *The isolation rate of the E. coli and K. pneumoniae from different samples. Among the 108 E. coli isolates, the highest number came from meat samples, 43.5%, followed by poultry environment and chicken faeces 40.7% and 15.7% respectively. Similarly, out of the 64 K. pneumoniae isolates obtained; 37.5% came from Chicken faeces while 32.3% each came from poultry environment and meat samples respectively.*

| Organism | Chicken faeces (n = 52) N (%) | Poultry environment (n = 134) N (%) | Meat (n = 86) N (%) | Total (n = 272) N (%) |
|---|---|---|---|---|
| *E. coli* | 17 (32.7) | 44 (32.8) | 47 (54.7) | 108 (39.7) |
| *K. pneumoniae* | 24 (46.2) | 20 (14.9) | 20 (23.3) | 64 (25.3) |
| Total | 41 (78.8) | 64 (47.8) | 67 (77.9) | 172 (63.2) |

***Table 2****: Isolation rate of the E. coli and K. pneumoniae from different samples.*

## Antimicrobial susceptibility

The results of the antimicrobial susceptibility testing are shown in table 3. Overall, the highest resistance rate was observed against cefotaxime (70.9%), followed by amoxicillin-clavulanic acid (66.3%) and ceftazidime (65.1%). The most active antibiotic against the isolates was imipenem, with a considerably low resistance rate of (13.9%). Considering sample sources and antibiotics resistance pattern; bacterial isolates from meat samples showed a slightly higher resistance rate when compared with samples from chicken faeces and poultry environment combined. For example, *K. pneumoniae* isolates from meat samples showed a resistance rate of 100%, 100%, 95%

against cefepime, cefotaxime, and amoxicillin-clavulanic acid respectively while isolates from chicken faeces and poultry environment had resistance rates of 72.7%, 72.7%, and 56.8% against the same antibiotics. A similar trend was observed in *E. coli* isolates from meat samples; 100% resistance to ceftazidime, cefotaxime (97.8%) and amoxicillin-clavulanic acid (95.7%); while *E. coli* from chicken faeces and poultry environment had a lower resistance rate, ceftazidime (26.2%), cefotaxime (39.3%) and amoxicillin-clavulanic acid (40.9%). However, there was no significant difference in the rate of resistance between *E. coli* and *K. pneumoniae* ($P > 0.05$).

| Antibiotics class | Antibiotics | Poultry and Environment | | Meat | | Total |
|---|---|---|---|---|---|---|
| | | *E. coli* N = 61 (%) | *K. pneumoniae* N = 44 (%) | *E. coli* N = 47 (%) | *K. pneumoniae* N = 20 (%) | N = 172 (%) |
| Carbapenems | IPM | 8 (13.1) | 8 (18.2) | 6 (12.8) | 2 (10.0) | 24 (13.9) |
| Cephalosporins | CEF | 26 (42.6) | 32 (72.7 | 25 (53.2) | 20 (100) | 103 (59.8) |
| | CAZ | 16 (26.2) | 31 (70.5) | 47 (100) | 18 (90.0) | 112 (65.1) |
| | CTX | 24 (39.3) | 32 (72.7) | 46 (97.8) | 20 (100) | 122 (70.9) |
| | FOX | 20 (32.7) | 30 (68.2) | 24 (51.1) | 8 (40.0) | 82 (47.7) |
| Penicillin | AUG | 25 (40.9) | 25 (56.8) | 45 (95.7) | 19 (95.0) | 114 (66.3) |
| Macrolide | E | 15 (24.6) | 22 (50) | 21 (44.7) | 9 (45.0) | 67 (38.9) |
| Fluoroquinolones | MFX | 9 (14.8) | 6 (13.6) | 29 (61.7) | 7 (35.0) | 51 (29.7) |
| Phenicols | C | 27 (44.3) | 28 (63.6) | 35 (74.5 | 18 (90.0) | 108 (62.8) |
| Aminoglycosides | CN | 26 (42.6) | 24 (54.5) | 36 (76) | 16 (80.0) | 102 (59.3) |

***Table 3:*** *Antibiotic resistance pattern of the E. coli and K. pneumoniae isolates.*

*Key: IMP: Imipenem; CEF: Cefpirome; CAZ: Ceftazidime; FOX: Cefoxitin; CTX: Cefotaxime; AMG: Amoxicillin-Clavulanic Acid; E: Erythromycin; MFX: Moxifloxacin; C: Chloramphenicol; CN: Gentamycin.*

## Phenotypic detection of ESBL producing isolates

Out of the 172 bacterial isolates identified as *E. coli* and *K. pneumoniae* in this study, 16.9% (29/172) were phenotypically characterized as ESBL producing isolates. These 29 isolates came from chicken faeces samples (7), poultry environment samples (10), and meat samples (12). Among the *E. coli* isolates 18.5% (20/108) were ESBL producers while 14.1% (9/64) were detected among the *K. pneumoniae* isolates. The distribution of the isolates according to samples sources (Table 4) shows that *E. coli* isolates that produced ESBL enzymes were phenotypically detected in chicken faeces samples (35.3%), poultry environment samples (13.6%), and meat samples (17.0%). Similarly, 4.2% of *K. pneumoniae* isolates from chicken faeces, 20% each from poultry environment and meat samples respectively were identified as ESBL producers by DDST.

| Organism | No. of Isolates from Chicken faces samples | No. of ESBL producers (%) | No. of Isolates from Poultry environment samples | No. of ESBL producers (%) | No. of Isolates from meat samples | No. of ESBL producers (%) | Total No. of Isolates | Total No. of ESBL producers (%) |
|---|---|---|---|---|---|---|---|---|
| *E. coli* | 17 | 6 (35.3) | 44 | 6 (13.6) | 47 | 8 (17.0) | 108 | 20 (18.5) |
| *K. pneumoniae* | 24 | 1 (4.2) | 20 | 4 (20.0) | 20 | (20.0) | 64 | 9 (14.1) |

***Table 4:*** *Distribution of ESBL producing isolates according to samples sources.*

## PCR detection of ESBL genes

Among the 29 isolates phenotypically characterized as ESBL producers, 12 were randomly selected (comprising both *E. coli* and *K. pneumoniae* from all samples sources) for PCR confirmation. The PCR assay showed that all the 12 isolates harbored at least one ESBL gene; $bla_{CTX-M}$, $bla_{TEM}$, or $bla_{SHV}$ (Figure 2A-2C). The three ESBL genes were all detected in 58.3% (7/12) of the isolates (Table 5). The most prevalent ESBL genes among the isolates was $bla_{CTX-M}$ detected in 91.7% (11/12) of the isolates, followed by $bla_{TEM}$ ,75% (9/12), and $bla_{SHV}$, 66.7% (8/12) respectively. All the ESBL producing isolates showed similar antibiotics resistance profiles with all the isolates being multidrug-resistant (i.e. resistance to three or more classes of antibiotics).

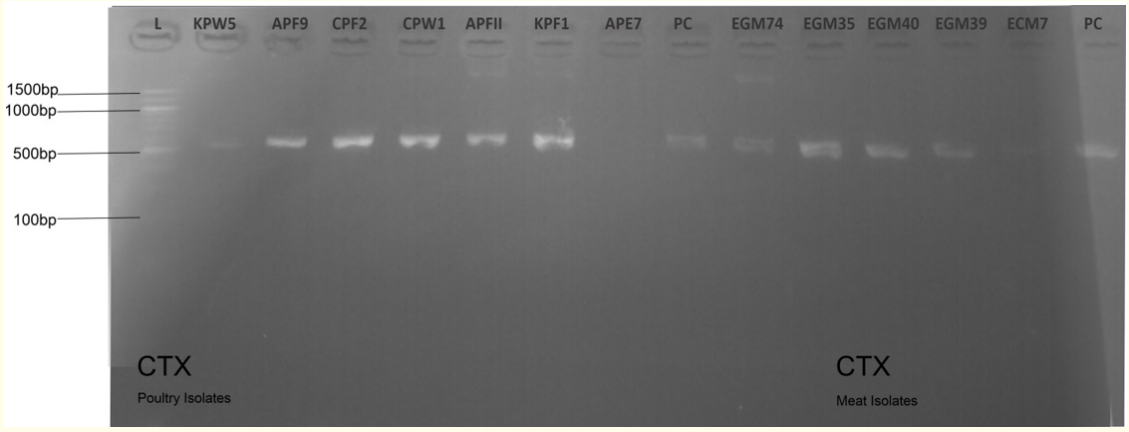


***Figure 2A:*** *Agarose gel showing PCR amplified product of blaCTX-M genes (band size 544 bp), lane L = 1 kb DNA ladder, lane PC = positive control, strains APE7 is blaCTX-M negative while all other strains are blaCTX-M positive.*

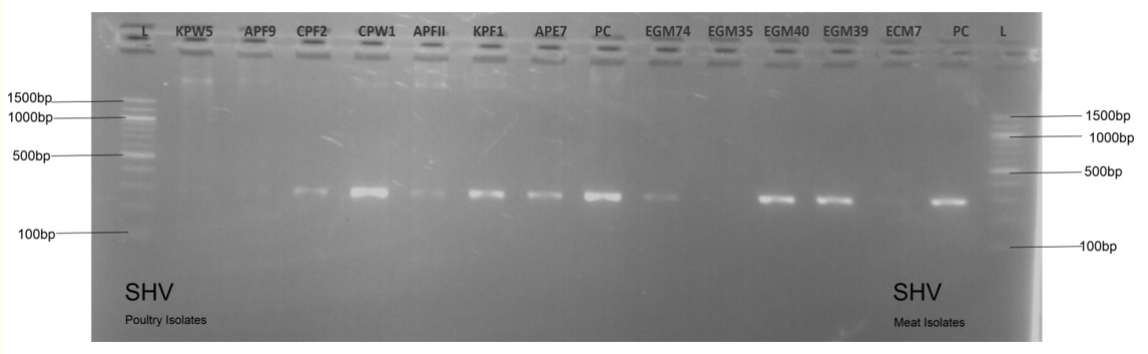


***Figure 2B:*** *Agarose gel showing PCR amplified product of blaSHV genes (band size 445 bp), lane L = 1 kb DNA ladder, lane PC = positive control, strains APF9, KPW5, EGM35 and ECM7 are blaSHV negative while all other strains are blaSHV positive.*

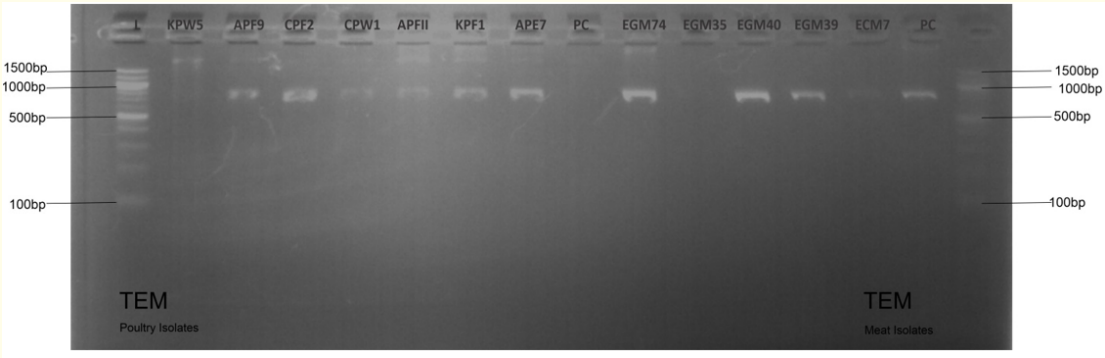


***Figure 2C:*** *Agarose gel showing PCR amplified product of blaTEM genes (band size 861bp), lane L = 1 kb DNA ladder, lane PC = positive control, strains KPW5, ECM7 and EGM35 are blaTEM negative while all other strains are blaTEM.*

| Isolate ID | Isolate Source | Isolate Name | Antibiotics Resistance Profile | ESBL Genes | | | Total number of ESBL Genes |
|---|---|---|---|---|---|---|---|
| | | | | $Bla_{CTX-M}$ | $bla_{TEM}$ | $bla_{SHV}$ | |
| KPW5 | Poultry Environment | *K. pneumoniae* | IMP, CEF, CAZ, CTX, AUG, FOX, C, CN | + | - | - | 1 |
| APF9 | Chicken Faeces | *E. coli* | IMP, CEF, CAZ, CTX, AUG, FOX, C, MFX | + | + | - | 2 |
| CPF2 | Chicken Faeces | *E. coli* | CEF, CAZ, CTX, AUG, FOX, C, CN | + | + | + | 3 |
| CPW1 | Poultry Environment | *E. coli* | IMP, CEF, CAZ, AUG, FOX, C, CN | + | + | + | 3 |
| APF11 | Chicken Faeces | *E. coli* | IMP, CEF, CAZ, CTX, AUG, FOX, C, CN | + | + | + | 3 |
| KPF1 | Chicken Faeces | *E. coli* | CEF, CAZ, CTX, AUG, FOX, C, CN | + | + | + | 3 |
| APE7 | Poultry Environment | *E. coli* | CEF, CAZ, CTX, AUG, FOX, MFX, C, E | - | + | + | 2 |
| EGM74 | Meat | *K. pneumoniae* | CEF, CAZ, CTX, AUG, FOX, C, CN, E | + | + | + | 3 |
| EGM35 | Meat | *E. coli* | CEF, CAZ, CTX, AUG, FOX, C, CN, E | + | - | - | 1 |
| EGM40 | Meat | *E. coli* | IMI, CEF, CAZ, CTX, AUG, FOX, C, E | + | + | + | 3 |
| EGM39 | Meat | *E. coli* | IMI, CEF, CAZ, CTX, AUG, FOX, C, E | + | + | + | 3 |
| ECM7 | Meat | *K. pneumoniae* | IMI, CEF, CAZ, CTX, AUG, FOX, C, E | + | - | - | 1 |

***Table 5:*** *Distribution of the ESBL genes among the isolates.*

*Key: IMP: Imipenem; CEF: Cefpirome; CAZ: Ceftazidime; FOX: Cefoxitin; CTX: Cefotaxime; AMG: Amoxicillin-Clavulanic Acid; E: Erythromycin; MFX: Moxifloxacin; C: Chloramphenicol; CN: Gentamycin.*

## Discussion

The impact of food animals such as poultry as a possible source of ESBL-producing Enterobacteriaceae and the spread of such strains into the food production chain cannot be overemphasized. Previous studies have reported that poultry might be the reservoir of ESBL pro-

ducing Enterobacteriaceae which can be transmitted to humans [13-15]. In this study, we investigated the prevalence of ESBL producing *E. coli* and *K. pneumonia* isolates in the food production chain using samples from Chicken faeces, poultry environment, and retail meat. Our data suggested the presence of high antibiotics resistant *E. coli* and *K. pneumonia* in all the samples sources; thus acting as a possible reservoir of antimicrobial-resistant bacteria which is a potential health risk to the human population.

The isolation rate of 62.8% for *E. coli* and 37.2% for *K. pneumoniae* observed in this study is higher than that of Ejikeugwu., *et al*. [16], which reported the isolation rate of 36.8% and 30.9% for *E. coli* and *K. pneumonia* in another part of Nigeria. However, our data is slightly similar to that of Eibacha., *et al*. [17] which reported an isolation rate of 56.8% for *E. coli* and 43.2% for *K. pneumoniae* in Ghana. The higher prevalence of *E. coli* when compared to *K. pneumoniae,* observed in this study, is in line with previous studies conducted in other parts of the world for example in Germany [18] and Thailand [19], which reported higher isolation rates of *E. coli* in samples collected from Chicken, poultry environment and retail meat. The occurrence of Enterobacteriaceae in the chicken and poultry environment is not abnormal due to the colonization of the gastrointestinal tract of animals with a variety of bacteria. However, since these pathogenic bacteria may enter the human food chain such as through retail meat as seen in this study and eventually lead to serious infections and transmission of antibiotic resistance traits, developing countries such as Nigeria should implement strict control policies that will reduce the spread of pathogenic bacteria in the food production chain [20].

High levels of resistance were observed in *E. coli* and *K. pneumoniae* isolates against the antibiotics tested in this study including the aminoglycosides, cephalosporins, macrolides, and fluoroquinolones. This may be due to the inadequate regulation and easy access to antibiotics for poultry farmers in Nigeria as against the developed countries where there is proper policy guiding antibiotic use in food production animals [21]. These data also confirm the previous reports of the emergence and dissemination of antibiotic-resistant Enterobacteriaceae in non-hospital environments [22,23]. The high resistance rate against fourth-generation cephalosporin, cefpirome (59.8%), and third-generation cephalosporins; ceftazidime (65.1), cefotaxime (70.9%) shown in this study are in line with other similar studies in Nigeria. For example, Ejikeugwu., *et al*. [16] reported more than 70% resistance while Otokunefor., *et al*. [24] reported ≥ 80% resistance rates against third-generation cephalosporins. However, contrary to the high resistance against imipenem (53.3%), reported by Ejikeugwu., *et al*. [16], we observed a considerable low resistance of 13.9% against imipenem in this study. The 59.3% resistance rate against gentamycin observed in this study is similar to the 55.6%, which Aworh., *et al*. [25] found in ESBL isolate from chickens and poultry environments in northern Nigeria and the 58.4% Trongjit., *et al*. [19] reported in Thailand. Another finding of this study is the higher resistance rate of 66.3% against penicillin compared with the result of Dsani., *et al*. [26] from Ghana who reported a resistance rate of 47% in *E. coli* isolates from raw meat.

In this study, we used both the DDST and PCR to investigate ESBL producing isolates. The phenotypic DDST showed that 16.9% (29/172) of the isolates were ESBL positive; PCR showed that all the twelve isolates subjected to the assay harbored one or more ESBL genes; this confirms the accuracy of our phenotypic test. The most prevalent ESBL gene among the isolates was $bla_{\text{CTX-M}}$ detected in 91.7% (11/12) of the isolates. This result is consistent with findings from other studies in Nigeria [25], Ghana [27], India [28], France [29] and England [30]; which showed a higher prevalence of $bla_{\text{CTX-M}}$ in Enterobacteriaceae isolates from poultry, retail meat, and environmental samples. In addition to $bla_{\text{CTX-M}}$, $bla_{\text{TEM}}$ and $bla_{\text{SHV}}$, which were also detected in this study, have also been reported to confer the ESBL phenotype in poultry isolates [4]. The production of ESBL enzyme by these isolates is worrisome because these pathogens have the exceptional ability to resist the antimicrobial onslaughts of other agents such as carbapenems which are last-line antibiotics used in clinical medicine.

## Limitation of the Study

This study has some limitations including the inability to carry out PCR assay on all the phenotypically detected ESBL producing isolates due to financial constraints. Also, the clonality of isolates could not be determined due to the lack of modern typing techniques such as multilocus sequence typing (MLST) or pulsed-field gel electrophoresis (PFGE).

## Conclusion

This study detected three ESBL genes, $bla_{CTX-M,}$ $bla_{TEM}$, and $bla_{SHV}$ in *E. coli* and *K. pneumoniae* isolates from the three samples sources investigated. This is worrisome considering the potential transmission of ESBL producers to humans via the food chain. The high numbers of antimicrobial-resistant bacteria observed in this study are of public health concern because this may represent a potential source of transmission to the human population. Our data support the scientific evidence from around the world, that the overuse or misuse of antibiotics in food animal production contributes significantly to the emergence and transmission of antimicrobial-resistant bacteria in the community. Our findings can serve as a guide for the development and establishment of antimicrobial resistance monitoring units in Nigeria to mitigate the further transmission of resistant strains in the community. To monitor the sources of emerging multidrug-resistant pathogens in Nigeria, we recommend adequate surveillance activities to be combined with a more stringently regulated use of antibiotics on local poultry farms and increased awareness of retail meat hygiene amongst the population.

## Funding Support

The authors received no funding for this study.

## Conflict of Interest

All authors declare no conflict of interest.

## Ethical Approval

All sampling procedure was by the guidelines of the National Health Research Ethics Committee, Nigeria (nhrec.net).

## Acknowledgments

We wish to thank the poultry farm owners for allowing the researchers to collect samples from their farms.

## Authors Contribution

CAN and CMN conceived and designed the study. CAN, PCU, KO and ESU conducted experiments. CAN and CMN analyzed data. CAN and CMN wrote the manuscript.

## Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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